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DEVELOPMENT OF IMMUNITY[1, 2]

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The study of the historical development of man and animals yields valuable information for a better understanding of anatomy and physiology. Immunity reactions in vertebrates have been found as far back as the cyclostomes, if only in rudimentary form [10, 11, 26]. From the historic-developmental point of view, individual ontogenesis reflects the phylogenesis of the vertebrates. Hence, we shall deal here with immunity development of the human individual. It is known that this immunity consists of numerous specific immunities against the various pathogenic agents and other antigens. The degree of these specific immunities of individuals is also variable and subject to constant changes depending on the varying supply of antigens. We may thus consider the sum total of these immunities as an immunity spectrum subject to constant modification by endogenous and exogenous effects.

An ontogenetic consideration of this spectrum permits establishment of a schematic order, a scale of immunity which may facilitate the understanding of immunity processes. The structure of such a schematic scale (Table 1) shows phagocytosis as the preliminary step, the so-called delayed reaction by cellular antibodies as the first and free antibodies as the second step.

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[1]With thanks to Prof. Dr. A. Wacker.
[2]Based on a lecture given at the Meeting of the Austrian Society for Microbiology and Hygiene, Graz, September 1964.

Table 1

Schematic Scale of Immunity

Steps	Kind of defense	Kind of cell	Kind of antigens	Pathology
Preliminary step	Phagocytosis	Microphages Macrophages	Small bodies Large, up to cell size	
1st step	Triggering of delayed reaction	Lymphocytes	Tb bacteria, viruses, foreign cells (soluble antigens)	Delayed reaction, granulomas, autoimmunity reactions
2nd step	19 S antibodies 7 S antibodies	Lymphocytes Plasma cells	Soluble antigens, bacteria, viruses, foreign cells	Anaphylaxis (autoimmunity reactions)

Preliminary Step: Phagocytosis

Phagocytosis cannot be classified among the immunity reactions without further consideration, for historically this is a very early process, seen even in unicellular organisms. In primitive multicellular organisms, phagocytosis of bacteria, their digestion and the regeneration of defects have been developed as protective processes which totally lack specificity, thus must be differentiated from immunity processes. In the higher vertebrates, phagocytosis represents a much more complicated process. We find two cellular forms here: 1. leukocytes with a polymorphic nucleus, called microphages, which envelop minute particles up to bacterial size, and 2. monocytes or macrophages, so large as to be able to swallow whole cells.

It has been known from numerous observations that monocytic phagocytosis may be strengthened by antibodies. These antibodies are called opsonins [32]. Their effect may be observed by comparing phagocytosis at the first immunization with that at a repeat immunization. Phagocytosis increases tremendously in the second case. Bacteria or protein floccules are taken up by the polymorphonuclear leukocytes. Large amounts of these cells are then swallowed up by the monocytes [2, 24, 32, 37, 44]. The biological role of this second stage of phagocytosis obviously consists in transporting the foreign material

to the regional lymph nodes and the spleen. There the antigen-filled monocytes exert a stimulatory effect on resting basophilic cells which will then multiply and differentiate into lymphocytes and plasma cells. Monocytic activity is thus specific in two directions: 1. by its increased takeup of specific antigens in the presence of the respective specific antibodies, 2. by transmitting the specific stimulatory effect of the antigen. The monocyte itself does not specifically respond to a specific irritant; it assumes the role of an intermediary. This justifies regarding phagocytosis as a preliminary step on the immunologic scale, a precursor of the immunity process proper.

First Step: Triggering of Delayed Reaction

In turning to these immunity processes, we would start from the immunologic condition of the newborn infant. He possesses the anatomic apparatus for immunity reactions, the spleen, lymph nodes, lymph follicles, bone marrow and thymus. However, this system is immature and non-exercised, for as a fetus in the womb the infant had been protected against exogenous antigen supply. Immunologic protection is thus assumed by the antibodies which have migrated from the mother's organism during the last months of pregnancy and have accumulated in the fetal serum [8, 38].

We now know that the maturation of the infant's own immunity system which proceeds under the protection of the maternal antibodies is directed by the thymus gland [6, 12, 19, 20]. This lympho-epithelial organ, developed from endodermal cells of the third gill sac, produces a hormone which is essential for the normal development of all immunity reactions. If the thymus of newborn animals is surgically removed, these animals will lack the normal immunity reactions to antigens and finally die under signs of lymphocytic insufficiency; but if the thymectomized animals receive a transplant from another newborn in a cell-tight chamber, these animals will develop normally [19, 20].

For our schematic immunity scale, it is of great importance that the avian thymus function is assumed by two organs, the thymus and another, equally lympho-epithelial organ which develops from endodermal epithelium in the terminal region of the rectum; this is called bursa Fabricii. If the bursa Fabricii is surgically removed in a newly hatched chick, this animal will be unable to produce free antibodies (see Table 2). However it can be sensitized with tuberculin and show the delayed reaction after intracutaneous tuberculin injection. The animal's tuberculin sensitivity can be transmitted to a normal

chicken by means of the former's lymphocytes. It is also possible to provoke experimental allergic encephalitis in chickens which lack the bursa Fabricii by injecting them with brain substance contained in Freund's adjuvant. This disease can be transmitted to other animals by the lymphocytes of the so sensitized animal. Chickens without bursa Fabricii are still capable of rejecting transplants from foreign chicken varieties by immunologic protective reactions. Hence, the following three reactions do not depend on bursa Fabricii functions: 1. delayed reactions of the tuberculin type; 2. production of experimental encephalitis, and 3. transplant rejection. If the thymus is surgically removed in a newly hatched chick, the animal cannot develop these three reactions, but it can form antibodies [6, 12, 14, 20, 21, 25]. Agammaglobulinemia of humans in which antibody formation is disturbed while the capacity for delayed reaction has been retained thus resembles the condition found in this bird after bursectomy (Table 2).

Table 2

Immunity Reactions in Birds and in Human Agammaglobulinemia

Reactions	Thymectomy	Bursectomy	Agammaglobulinemia
Antibody formation	+	-	-
Delayed reaction	-	+	+
Experimental encephalitis	-	+	
Transplant rejection	-	+	

All these findings justify drawing a line between antibody formation and the other reactions. The prototype of the other reactions is the delayed reaction, i.e., that which can be triggered in the tuberculous animal by an injection of tuberculin [1]. We elicit the capacity for delayed reaction by immunizing with weak antigens or very low doses of stronger antigens. If we immunize, e.g., a guinea pig with a very low dose of diphtheria antigen, antibodies will probably appear in this animal after about four-six weeks only; but, if this animal is injected after two weeks with a low intracutaneous dose of diphtheria antigen, a typical delayed reaction is obtained. The capacity for delayed reaction thus precedes that for antibody production [1, 18]. We have, therefore, assigned this immunity reaction to the first step, i.e., the beginning of immunity development, and the capacity for antibody formation and anaphylactic reaction to the second step.

Anaphylactic and delayed reaction will appear when an organism has been sensitized to an antigen and comes into

renewed contact with the same antigen. We shall make mention of the anaphylactic reaction only in passing: it appears within minutes and is based on the reaction of the antigen with free antibodies in serum or tissue fluid. Upon intracutaneous injection, rapid reddening and edema around the injection site is observed. Increased penetrability of vessels is characteristic of the anaphylactic reaction.

The prototype of delayed reaction is the tuberculin reaction [1]. If a tuberculous guinea pig is injected intracutaneously with a low tuberculin dose, slight reddening and edema appear not earlier than 4-6 hours after the injection; the reaction reaches its climax after 24-48 hours to recede slowly afterwards. Histologic examination of the reaction site, particularly the area around the veins, reveals dense monocytic infiltrates. Mitoses are remarkably numerous and point towards lively cell multiplication. Increased capillary permeability with edema formation is not a part of the normal picture of delayed reaction [1, 45]. The essential difference between delayed and anaphylactic reaction may be found in the apparent lack of free antibody participation in the delayed reaction, for we cannot transmit tuberculin sensitivity of a sensitized animal to a normal one by means of cell-free serum. This can be done only if we introduce the leukocyte fraction of the blood or a cellular suspension of spleen, lymphocytes, bone marrow or abdominal exudate into the normal animal [1, 31]. This recipient or host of the transmitted cells remains tuberculin-sensitive for the time during which the donor cells survive in his body. If we proceed with such cell transmission between two inbred experimental animals belonging to the same strain, the transmitted sensitivity to tuberculin may be retained for several weeks if the number of donor cells was sufficiently high. If we transmit such cells to alien species, tuberculin sensitivity will last only a few days, due to the considerably lesser survivability of the cells introduced into the alien medium. The transmitted cells will only trigger the delayed reaction. The dense cellular infiltrates are mainly host cells [31], i.e., they reflect a secondary reaction in the recipient. Thus, e.g., the underdeveloped immunity system of the newborn will initially be incapable of delayed reaction [1, 38]. In respect to exchange transfusion in the newborn, this involves the transmission of so many white blood cells that not only specifically sensitized immune cells but also a sufficient number of other lymphocytes and monocytes will be present to achieve a delayed reaction [1]. If exchange transfusion is performed in a BCG-vaccinated infant, adverse reactions may occur in the area of the vaccinal infection foci [39].

Experimental allergic encephalitis is a specific case of delayed reaction; there, immunization with brain antigen in the

primary cause. Histologic findings consist of perivenous focal encephalitis, i.e., cellular infiltrations around the cerebral veins. Migration of monocytes into the perivenous tissue is accompanied by extensive destruction of the sheaths covering the marrow-containing nerve fiber. There is a very close relationship between monocytal infiltrate and myelin destruction [1].

It has recently been stressed that development of tuberculin hypersensitivity and the local reaction following smallpox vaccination are closely related [29, 30]. In cases of agammaglobulinemia, a perfectly normal course of local reaction is seen after the first smallpox vaccination. Thus, no serum antibodies are required for this reaction. This is another reason for assuming, in analogy to conditions for experimental encephalitis, that postvaccinal encephalitis is pathogenetically determined by a kind of delayed reaction. In clinical agammaglobulinemia, the lack of antibody formation is much less serious for protection against viral disease than for the course of bacterial infections. It seems that the first step of immunity, delayed reaction, is often sufficient to combat virus infections.

Delayed reaction assumes an important role in transplant rejection. It has been shown in many experiments that prompt transplant rejection is possible without serum antibodies acting against the transplant cells. Histologic examination reveals dense monocytic or lymphocytic infiltrates at the boundaries of the transplants. These same signs were seen in tumor transplants. Many authors consider the density of cellular infiltrates as a criterion of the host's protective resources against the tumor transplant and thus a favorable prognostic sign [16].

It is characteristic for all these reactions that they result in certain injuries to the host. These consist in focal reactions at the site of antigen injections, of endogenous antigenic foci or transplants; local tissue reactions may reach the necrotic stage if the dose of antigen available for the reaction is sufficiently high [1].

It is assumed that delayed reactions are among the leading pathogenic factors responsible for the majority of auto-allergic or auto-immune diseases. In, e.g., experimental allergic thyroiditis, a chronic inflammation of the thyroid, the severity of clinical signs parallels the capacity for delayed reaction against thyroid extract rather than the antibody level [17]. On the contrary, in this and other syndromes, the level of the antibody titer appears to be inversely proportional to the

degree of injury suffered by the patient. Injury inflicted on tumor transplants by the host, conceived as delayed reaction, also appears to decrease with an increase in the tumor-specific antibody level [4, 16, 36]. This relationship, apparently, also applies to experimental encephalitis, for the encephalitic reaction will fail to appear if the experimental animal possesses serum antibodies against the cerebral antigen [27].

These recent observations suggest the conclusion that allergicallergic encephalitis is based on a delayed reaction which may be inhibited by the early development of serum antibodies. As early as 30 years ago, Herzberg related postvaccinal encephalitis to a disturbed vaccinal process; he considered a prolonged stage of vaccinal viremia as a sign for such disturbance [13]. We would add that the rapid disappearance of the antigen from circulation following antigen injection is the first and most sensitive sign of the start of antibody formation against the specific antigen; therefore, prolonged viremia would indicate that antibody formation takes longer than usual and thus keeps the respective organism longer at the first step of immunity development.

I would want to remark on the still controversial subject of delayed reaction pathogenesis that the old concept of cellular antibodies still offers the best explanation for these observations. The immune cells which transmit the delayed reaction must have the capacity for binding antigens with antibody-like structures. Such structures, capable of binding antigens, have been determined in sensitized peritoneal cells or extracts from such cells [9, 22]. When such cells come into contact with antigen, specific bonding reactions probably occur which result in the liberation of toxic products. If this happens in the immediate neighborhood of the sensitized cells, it would probably result in severe injury or destruction of these cells [15]; it would also show which irritations cause host reactions resulting in such dense monocytic infiltration. The dense cellular infiltrates and the circulatory disturbances caused by them have been considered the pathogenic factor for the necroses developing in focal tuberculin reactions [1]. Circulatory disturbances are obviously equally capable of disrupting blood supply to the transplant or tumor transplant and thus initiate necrosis.

Second Step: Antibody Formation

The second step on the schematic scale shows the 19 S and 7 S antibodies (Table 1). The 19 S antibodies have 19 Svedberg units in the ultracentrifuge and a molecular weight of about one million [34]. The 7 S antibodies have only 7 Svedberg

units and a molecular weight of about 150,000-160,000.

A simple method exists for distinguishing between the large and small antibodies. The 19 S antibodies are destroyed by the addition of mercaptoethanol while the 7 S antibodies are resistant to this substance [40, 41].

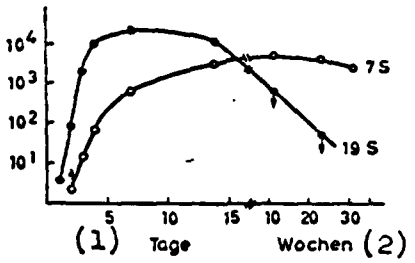

Fig. 1. 19 S and 7 S Antibody Curves Following a Single Intravenous Injection of a High Poliovirus Dose into Rabbits (according to Shevag and Mandel [41]).

1 -- Days; 2 -- Weeks.

The appearance of both kinds of antibodies may be provoked by the same antigen injection. Fig. 1 shows, as an example, the shape of the 19 S and 7 S antibody curves in the rabbit after a single vaccination with a high poliovirus dose. The 19 S antibodies appear earlier and disappear soon. The 7 S antibodies appear later and are apparently the kind of antibodies which we find in stable longlasting immunity [41]. If we consider that both antibody forms appear bivalent with respect to antigen binding force, the 7 S antibody, with its much lower molecular weight, definitely appears as the more economic form. Both forms of antibodies are found in vertebrates, from fish to man, but in the line from fish to bird the capacity for 7 S antibodies is developed in a lesser degree [7, 10, 11, 35, 42].

In mammals, weak antigens or low doses of strong antigens will lead only to the production of 19 S antibodies. Immunization of rabbits with Salmonella O antigens results in a much lower antibody titer than that with H antigens. It has recently been reported from the Burnet Institute that Salmonella O antigens will stimulate only 19 S antibody production in rats while H antigens will provoke the formation of both antibodies [23]. Other authors were able to produce 7 S antibodies in rabbits with Typhus O antigen; however, very high doses of antigen had to be used, and the effect was not regularly reproducible [3, 5, 28, 43]. A team of investigators at Burnet's Institute applied a very subtle technique to examine the antibody production of individual cells against Salmonella H antigens and

found that only 19 S antibodies will appear during the first five days while both types are produced on the sixth and ninth day; at a later stage, these investigators found only 7 S antibodies. They believe that some cells will produce only 19 S, others only 7 S antibodies, but that the same cells are capable of producing successively and, at an intermediary stage, also simultaneously, both forms of antibodies [23].

In regard to associating antibody production with certain cellular forms, no sharp differentiation exists. It is true that the majority of 19 S antibody producers have the lymphocytic, those of 7 S the plasma cell form, but there are many exceptions [33].

Based on the preliminary observations reported so far, we may classify 19 S antibody production as an early stage of antibody formation in both the phylogenetic and ontogenetic sense and possibly subdivide the second step on our schematic scale, that of the antibodies.

What is the utility of such a schematic scale? It may be used for teaching purposes, in immune biology and as a qualitative scale for evaluating immunologic reactions. We recognize the limited usefulness of the delayed reaction for stopping the spread of tuberculosis, in the protection against viral infections, and in the rejection of foreign cells. We also recognize the 7 S antibody as the most perfect product of the immunity system. It is perfect in regard to economy of production, ability for rapid multiplication in secondary reactions, the strength of its antibody bonds. The same applies to its specific producer, the plasma cell, which is to be considered the functionally best differentiated immune cell.

Hence, we must demand that a vaccine be capable of stimulating the production of 7 S antibodies in the vaccinated individual. If the Salmonella O antigens which are of prime importance for vaccinal protection against typhoid and paratyphoid can bring about usually nothing more than 19 S antibody production, this would constitute a simple criterion that the required optimum has not yet been reached. The situation is even worse for tuberculosis, for here protection stops at the first step, that of delayed reaction.

This schematic scale leads towards specific questions regarding the mode of action by which some antigens are capable of stimulating the immunity system into producing 7 S antibodies while other antigens are incapable of such an effect. Since these processes involve the participation of certain other cellular proliferative processes leading to the development of plasma cells, it is possible that nonspecific

stimulation may play a role in cellular proliferation or cell differentiation. In this connection, we would mention the non-specific stimulatory effect of adjuvants; particularly Freund's adjuvant seems to induce considerable cell multiplication in the area of the injection site and the regional lymph nodes. The problem of improving the vaccinal protective effect against typhoid, tuberculosis and similar diseases will probably be solved only if we are able to develop optimal adjuvants. This form of scruting also shows that the ontogenetic schematic scale is probably apt to raise more new questions than answer old ones.

Summary

A schematic scale showing the development of individual immunity from the ontogenetic point of view has been drawn up, based on the evaluation of recent immuno-biological studies. Phagocytosis is considered a preliminary step, the triggering of delayed reaction as the first, antibody formation as the second step. Delayed reaction is discussed and its importance for immunopathology explained. Antibody formation starts with 19 S antibodies and proceeds to the 7 S antibodies. The schematic scale supplies a basic structure for the science of immuno-biology and new points of reference for the evaluation of vaccines.

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